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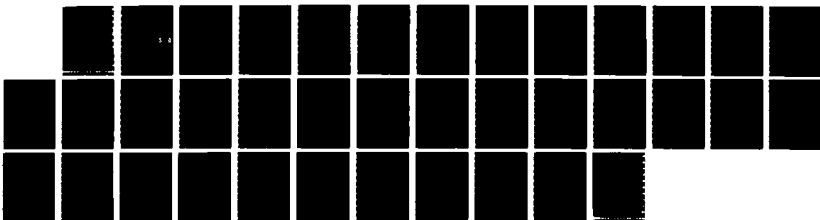
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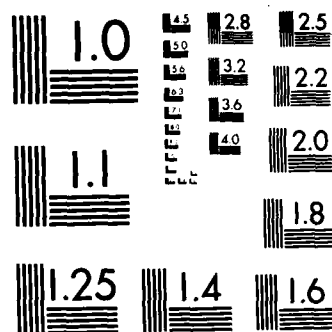
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CHEMICAL PREPARATION LABORATORY
FOR IND CANDIDATE COMPOUNDS
ANNUAL REPORT



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Chemical Preparation Laboratory for IND Candidate Compounds

Annual Report

by

E.M. Schubert, Ph.D.

January 21, 1986

(January 17, 1985 - January 16, 1986)

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-85-C-5071

Pharm-Eco Laboratories, Inc.
2355 Chain Drive, Simi Valley, California 93065

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No 0704-0188
Exp Date Jun 30, 1986

1a REPORT SECURITY CLASSIFICATION Unclassified			1b RESTRICTIVE MARKINGS		
2a SECURITY CLASSIFICATION AUTHORITY			3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution unlimited		
2b DECLASSIFICATION/DOWNGRADING SCHEDULE					
4 PERFORMING ORGANIZATION REPORT NUMBER(S)			5. MONITORING ORGANIZATION REPORT NUMBER(S)		
6a. NAME OF PERFORMING ORGANIZATION Pharm-Eco Laboratories, Inc.	6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION			
6c. ADDRESS (City, State, and ZIP Code) 2355 Chain Drive Simi Valley, CA 93065		7b. ADDRESS (City, State, and ZIP Code)			
8a. NAME OF FUNDING/SPONSORING ORGANIZATION U. S. Army Medical Research and Development Command	8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER DAMD17-85-C-5071			
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick, Frederick, MD 21701-5012		10. SOURCE OF FUNDING NUMBERS			
		PROGRAM ELEMENT NO. 63763A	PROJECT NO. 3M263 763D807	TASK NO. AD	WORK UNIT ACCESSION NO. 036
11. TITLE (Include Security Classification) Chemical Preparation Laboratory for IND Candidate Compounds					
12 PERSONAL AUTHOR(S) Ernst M. Schubert, Ph.D.					
13a. TYPE OF REPORT Annual	13b. TIME COVERED FROM 1/17/85 TO 1/16/86	14. DATE OF REPORT (Year, Month, Day) Jan. 21, 1986		15. PAGE COUNT 33	
16. SUPPLEMENTARY NOTATION					
17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)		
FIELD	GROUP	SUB-GROUP			
07	05		Nucleosides, antivirals, selenazoles, triazoles, alkaloids, formycin B, C-nucleosides, ribavirin		
06	03				
19. ABSTRACT (Continue on reverse if necessary and identify by block number)					
<p>Six compounds were synthesized during the reporting period: 3-β-D-ribofuranosyl-1(H)pyrazolo[4,3-d]pyrimidin-7(6H)thione (Thioformycin-B); 9-β-D-ribofuranosyl-purine-6-thiocarboxamide; 3-bromo-4-chloro-pyrazolo[3,4-d]pyrimidine; 2-β-D-ribofuranosylselenazo-4-carboxamide; cis, trans 3,6-diethoxy-tetrazadiphosphorine-3,6-disulfide; 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (Ribavirin). Four compounds remain under investigation: tri-O-acetyl(±)lycoricidine; 5'-O-(1-methyl-1,4-dihydronicotinoyl)-1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide; 5'-deoxy-5'-(N-1,4-dihydronicotinamidy)-1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide; 2',3',5-tri-O-(1-methyl-1,4-dihydronicotinoyl)-1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide.</p>					
20 DISTRIBUTION/AVAILABILITY OF ABSTRACT <input type="checkbox"/> UNCLASSIFIED/UNLIMITED <input checked="" type="checkbox"/> SAME AS RPT <input type="checkbox"/> DTIC USERS			21 ABSTRACT SECURITY CLASSIFICATION		
22a NAME OF RESPONSIBLE INDIVIDUAL Mrs. Judy Pawlus			22b TELEPHONE (Include Area Code) 301-845-6349	22c OFFICE SYMBOL SGRD-RMI-S	

I. SUMMARY

The syntheses of ten target compounds have been examined during this annual period. The preparations of six of the ten target compounds were completed, and the compounds were transferred to USAMRIID, Department of Antiviral Studies. The syntheses of the four remaining target compounds, three of which are unreported in the chemical literature, are being further investigated.

The following target compounds were synthesized during the reporting period: 3- β -D-ribofuranosyl-1(H)pyrazolo[4,3-d]pyrimidin-7(6H)thione (Thioformycin-B), AVS 52; 9- β -D-ribofuranosyl-purine-6-thiocarboxamide, AVS 79; 3-bromo-4-chloro-pyrazolo[3,4-d]pyrimidine, AVS 222; 2- β -D-ribofuranosyl-selenazo-4-carboxamide, AVS 253; cis,trans 3,6-diethoxy-tetrazadiphosphorine-3,6-disulfide, AVS 593; 1- β -D-ribofuranosyl-1,2,4-triazole-3-carboxamide (Ribavirin), AVS RIB.

The syntheses of the following target compounds remain under investigation, and their preparations are continued: tri-Q-acetyl(\pm)lycoricidine, AVS 360; 5'-Q-(1-methyl-1,4-dihydronicotinoyl)-1- β -D-ribofuranosyl-1,2,4-triazole-3-carboxamide (Prodrug ester), AVS XXX; 5'-deoxy-5'-(N-1,4-dihydronicotinamidyl)-1- β -D-ribofuranosyl-1,2,4-triazole-3-carboxamide, AVS SPEC; 2',3',5'-tri-Q-(1-methyl-1,4-dihydronicotinoyl)-1- β -D-ribofuranosyl-1,2,4-triazole-3-carboxamide, AVS TRI.

II. FOREWORD

All information in this report is the property of the U.S. Army Medical Research and Development Command. The contractor retains no copyright or patent rights.

All target compounds reported herein were prepared in strict compliance with "Current Good Manufacturing Procedures" (CGMP) guidelines. All intermediates and final products unreported in the chemical literature were fully characterized by elemental and spectral analyses.

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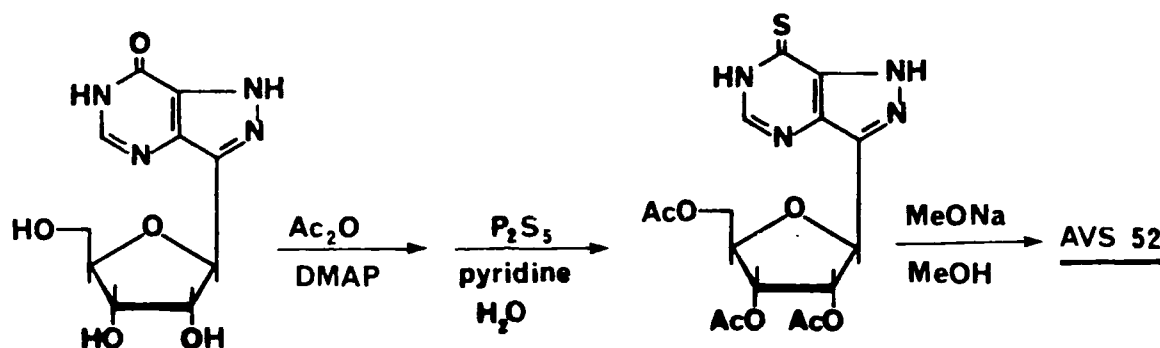
III. CUMULATIVE LIST OF COMPOUNDS COMPLETED AND DELIVERED TO U.S. ARMY
 MEDICAL RESEARCH INSTITUTE OF INFECTIOUS DISEASES (USAMRIID)
JANUARY 1, 1985 TO DECEMBER 31, 1985

<u>No.</u>	<u>Compound</u>	<u>Amount</u>	<u>PC No.</u>
AVS 52	3- β -D-ribofuranosyl-1(H)pyrazolo[4,3-d]- pyrimidin-7(6H)thione (Thioformycin-B)	10.2 g	PC 0907
AVS 79	9- β -D-ribofuranosyl-purine-6-thiocarboxamide	20.0 g	PC 0960
AVS 222	3-Bromo-4-chloropyrazolo[3,4-d]pyrimidine	63.1 g	PC 1040
AVS 253	2- β -D-ribofuranosylselenazo-4-carboxamide	28.3 g	PC 0954
AVS 593	cis,trans 3,6-diethoxy-tetrazadiphosphorine- 3,6-disulfide	10.15 g	PC 0986
AVS RIB	1- β -D-ribofuranosyl-1,2,4-triazole-3- carboxamide (Ribavirin)	2.0 Kg	PC 1211

IV. PROCEDURES FOR TARGET COMPOUNDS DELIVERED TO USAMRIID
from January 1, 1985 to December 31, 1985

A. 3-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-1(H)pyrazolo[4,3-d]-pyrimidin-
(6H)one, (Tri-O-acetylformycin-B), AVS 52

Synthetic Procedure:



EXPERIMENTAL

3-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-1(H)pyrazolo[4,3-d]pyrimidin-
7(6H)one, (Tri-O-acetylformycin-B): A mixture of Formycin-B (7.95 g, 29.66 mmol), 4-dimethylaminopyridine (200 mg) and acetic anhydride (200 mL) was stirred under anhydrous conditions at room temperature for 48 hours. Acetic anhydride was evaporated under reduced pressure and the residue was coevaporated with ethanol (1 x 100 mL). The syrupy material was refluxed gently for 30 minutes with ethanol-water (80:20, 150 mL) and cooled in an ice bath. The crystalline material was filtered and washed with water. Yield 8.0 g. The mother liquor was concentrated and cooled, the resulting crystalline material was filtered and washed with water to yield 2.0 g. An additional 0.2 g of the product was recovered from the above filtrate. The combined yield was 10.2 g (87.25%); m.p. 168°C (Lit. m.p. 168°C).

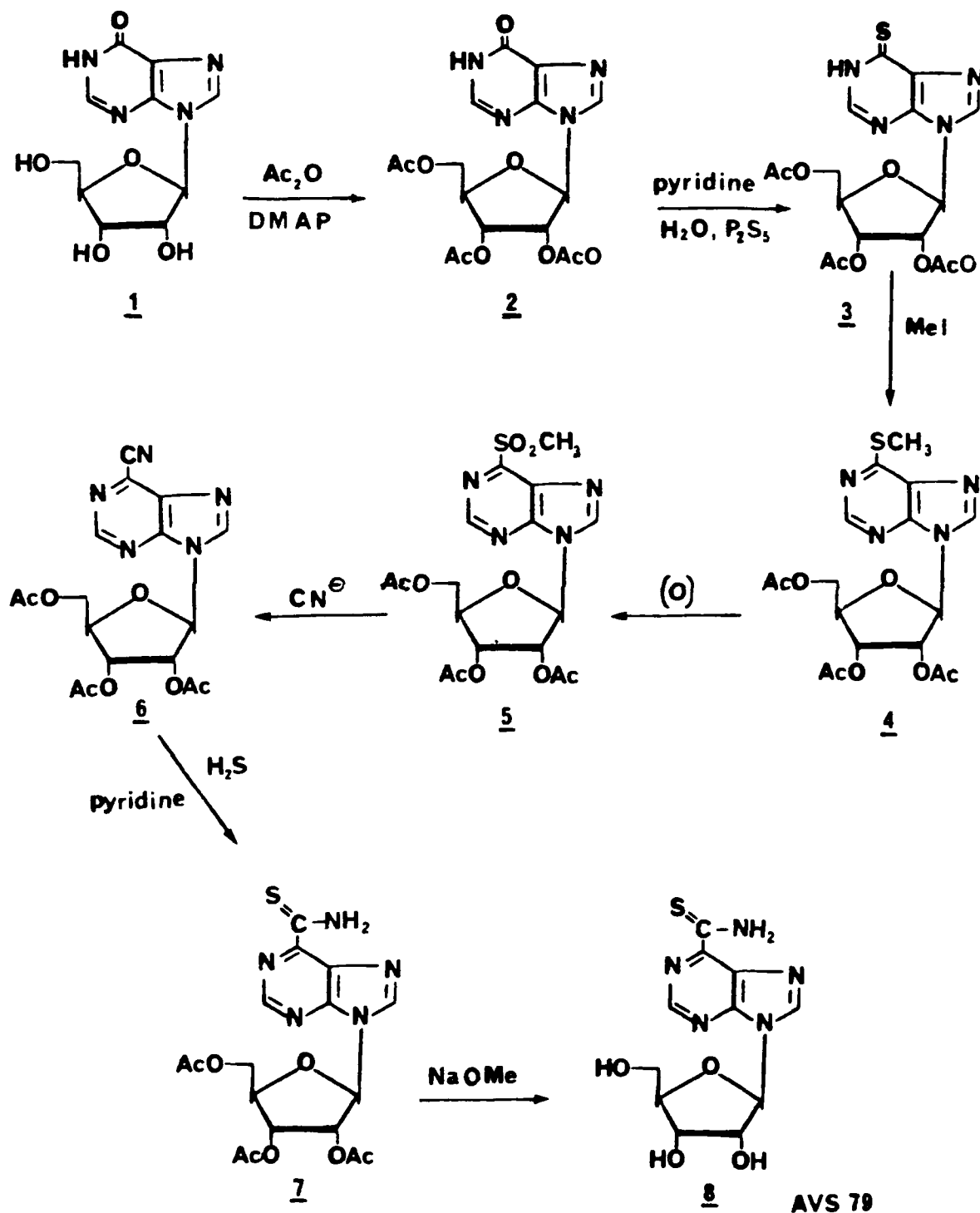
3-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-1(H)pyrazolo[4,3-d]pyrimidin-
7(6H)thione, (Tri-O-acetylthioformycin-B): To a well-stirred mixture of tri-O-acetylformycin-B (10.0 g, 25.4 mmol) and pyridine (350 mL), phosphorus pentasulfide (25 g, 56.0 mmol) was added. Water (3.7 mL, 200 mmol) was added dropwise and the reaction mixture was refluxed for 4.5 hours in an oil bath (bath temp. 135-40°C). The reaction mixture was cooled, chilled in an ice-water bath, and the supernatant liquid was decanted. The residue in the flask was carefully added to boiling water (500 mL). The previously decanted liquid was evaporated to a syrup and added slowly to the above boiling water while stirring. The reaction mixture was cooled and extracted with chloroform (3 x 300 mL). The chloroform layer was washed with saturated sodium chloride solution (2 x 400 mL) and the organic layer was dried over anhydrous sodium sulfate. Evaporation of the solvent gave a yellow foam. Yield 10.3 g (99%). m.p. 110°C.

3- β -D-Ribofuranosyl-1(H)pyrazolo[4,3-d]pyrimidin-7(6H)thione, (Thioformycin-B): Tri-O-acetylthioformycin-B (10.0 g, 24.4 mmol) was dissolved in anhydrous methanol (200 mL) and the pH of the solution was adjusted to 9-10 by adding sodium methoxide solution. The reaction mixture was stirred at room temperature for 4 hours, neutralized with Amberlite IR-120 H⁺ resin and filtered. The resin was washed with methanol (4 x 30 mL) and the filtrate was evaporated to yield a yellow solid, which was crystallized from methanol-water (9:1). Yield 6.0 g (86%); m.p. 233-234°C (Lit. m.p. 233°C).

Remark: Starting with 9.85 g Formycin-B, an identical second run produced 7.2 g Thioformycin-B. The two batches were combined to give a total yield of 11.0 g of analytically pure Thioformycin-B.

B. 9-β-D-Ribofuranosylpurine-6-thiocarboxamide, AVS 79

Synthetic Procedure:



EXPERIMENTAL

9-(2,3,5-Tri-O-acetyl- β -D-ribofuranosyl)hypoxanthine (Tri-O-acetyl-inosine) (2): A mixture of inosine (65.0 g, 0.242 mol), acetic anhydride (750 mL) and 4-N,N-dimethylaminopyridine (1.0 g) was stirred under anhydrous conditions at room temperature for 24 hours. The reaction mixture was heated in a water bath (bath temp. 75°) for 15 minutes. Unreacted acetic anhydride was evaporated under reduced pressure (bath temperature 40°C). The residue was carefully treated with methanol (300 mL) and evaporated to dryness. The white residue was triturated with ethanol (600 mL) and filtered. The white solid was washed with ethanol (2 x 100 mL), and air dried to give 94.7 g (99.7%) of 2. m.p. 244-245° (Literature m.p. 244°). All the spectral data matched reported values.

9-(2,3,5-Tri-O-acetyl- β -D-ribofuranosyl)-6-mercaptapurine (Tri-O-acetylthioinosine) (3): To a well stirred mixture of 2 (93.0 g, 0.236 mol) and pyridine (3570 mL), phosphorus pentasulfide (220.0 g, 0.49 mol) was added in one portion. Water (35.5 mL, 1.97 mol) was added slowly over a period of 15 minutes and the reaction mixture was refluxed for 7 hours. The reaction mixture was cooled to room temperature and a clear liquid was decanted from the thick viscous residue. This residue was slowly poured into boiling water (500 mL) while stirring. The previously decanted top layer was evaporated under reduced pressure to a thin syrup and added slowly to the boiling aqueous solution while stirring. After boiling for 30 minutes the reaction mixture was cooled and the solid which separated was filtered, washed with ice water (3 x 300 mL) and dried in air to yield 75.5 g (77.75%) of 3. m.p. 235-236°(dec). The structural identity of 3 was confirmed by spectral analyses.

9-(2,3,5-Tri-O-acetyl- β -D-ribofuranosyl)-6-methylthiopurine (4): To a well stirred mixture of 3 (73.0 g, 0.178 mol) and dimethylformamide (350 mL) in a three-necked flask fitted with a mechanical stirrer and a dropping funnel was added anhydrous potassium carbonate (28.0 g, 0.202 mol). Methyl iodide (57.0 g, 0.402 mol) was added slowly through the dropping funnel over 25 minutes. After stirring for 4 hours at room temperature, the reaction mixture was poured into ice-water (1000 mL) and extracted with ethyl acetate (3 x 400 mL). The organic layer was washed with saturated sodium chloride solution (2 x 400 mL), dried over anhydrous sodium sulfate and evaporated under reduced pressure. The syrupy residue was co-evaporated with ethyl acetate (2 x 200 mL) and the residue was kept under vacuum to form a foam which was homogeneous by TLC. Yield of 4: 63.1 g (83.5%). Spectral data of 4 agree with structural assignments.

9-(2,3,5-Tri-O-acetyl- β -D-ribofuranosyl)-6-methylsulfonylpurine (5): Method A: A solution of 4 (60.37 g, 0.142 mol) in glacial acetic acid (1800 mL) was maintained at about 0°C while potassium permanganate (44.0 g, 0.278 mol) was added slowly over 1 hr. The flask was removed from the cooling bath and the reaction mixture was stirred at room temperature for 4 hours. The dark colored reaction mixture was poured into water (3000 mL), and saturated with sodium chloride. After extraction with ethyl acetate (5 x 500 mL), the organic layer was washed with water (3 x 1000 mL), aqueous sodium bicarbonate solution (10%) (2 x 1000 mL), and dried over sodium sulfate. Evaporation of the solvent gave

36.46 g (67.5%) of 5 as a white foam. The product was homogeneous by TLC and its spectral data agreed with its structure. The product was used for further reaction without purification. Since Method A involved laborious workup procedures and gave poor yields, the reaction was modified by replacing potassium permanganate with m-chloroperbenzoic acid as the oxidizing agent.

Method B: To a solution of 4 (24.0 g, 0.052 mol) in dichloromethane (625 mL) m-chloroperoxybenzoic acid (20.0 g, 0.11 mol) was added in one portion and the reaction mixture was stirred at room temperature for 2 hours. TLC indicated the completion of the reaction. The reaction mixture was diluted with dichloromethane (300 mL) and washed carefully with saturated sodium bicarbonate solution to remove m-chloroperoxybenzoic acid and unreacted m-chloroperoxybenzoic acid. The organic layer was dried over sodium sulfate and evaporated under reduced pressure to give 5 as a white foam. Yield 24.0 g (93%). This product was identical to the one obtained by method A.

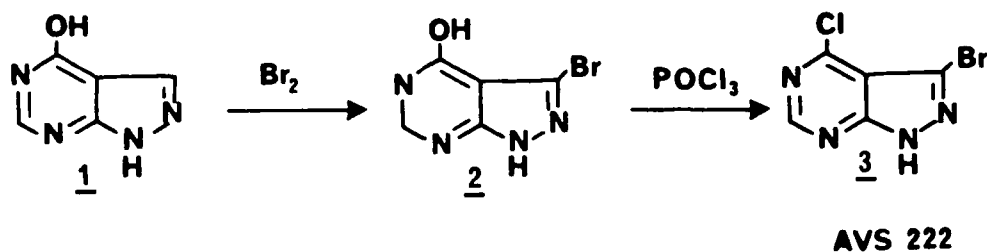
9-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-purine-6-carbonitrile (6)¹: Finely powdered potassium cyanide (8.5 g, 0.130 mol) was added to a well-stirred solution of 5 (35.8 g, 0.0785 mol) in dimethylformamide (205 mL), and stirring was continued at room temperature for 3.5 hours under anhydrous conditions. TLC indicated the presence of some unreacted starting material. An additional 2.0 g of powdered potassium cyanide was added to the reaction mixture and stirring was continued overnight. The dark colored solution was poured into water (1000 mL), neutralized with acetic acid (4 mL), extracted with ethyl acetate (4 x 300 mL) and the organic layer was washed with saturated sodium chloride solution (3 x 2000 mL). The organic layer was dried over sodium sulfate and evaporated to give 6 as a tan colored foam. Yield 26.3 g (83.13%). The spectral data of the product agreed with structural assignments. The compound was homogeneous by TLC and it was used in the next step without further purification.

9-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-purine-6-thiocarboxamide 7: A solution of 6 (25.0 g, 0.062 mol) in pyridine (1000 mL) was cooled in an ice-salt bath for 30 minutes, and a slow stream of hydrogen sulfide gas was passed through it for 1 hour. The bath was removed and the reaction mixture was stirred at room temperature for 2 hours. TLC of the reaction mixture indicated the completion of the reaction. A steady stream of nitrogen gas was passed through the reaction mixture to drive off unreacted hydrogen sulfide, then the pyridine was evaporated under reduced pressure. The residue was co-evaporated with toluene (2 x 200 mL). The dark foam was then coated onto silica gel (70 g) and loaded onto a column packed with silica gel in chloroform. The column was eluted with chloroform and the fractions AVS 79 containing compounds with identical R_f values were combined. After evaporation 7 was obtained as an orange form. Yield 24.6 g (90.75%). Spectral data of the product were in agreement with reported values.

9- β -D-Ribofuranosylpurine-6-thiocarboxamide (8)²: To a stirred solution of 7 (24.0 g, 0.055 mol) in methanol (700 mL) methanolic sodium methoxide solution (1N) was added to adjust the pH to 9. The reaction mixture was stirred at room temperature for 4.5 hours, at the end of which TLC showed the completion of the reaction. Glacial acetic acid was added to the reaction mixture to adjust the pH to 4. Charcoal (10.0 g) was added and the mixture was stirred at room temperature for an hour, then filtered through a Celite bed. The celite bed was washed with methanol until the washings were colorless. The filtrate was concentrated to 200 mL and allowed to stand at room temperature overnight. The light orange, fine crystals were filtered to yield 9.5 g of 8. The mother liquor was concentrated and cooled to give an additional 3.2 g of the product. The combined yield was 12.7 g (74.2%), m.p. 169°C (Lit. m.p. 167-169°C).

Following this procedure twice, a total of 21.0 g of final product was obtained. The spectral data of the combined batches were in agreement with reported values. Elemental analysis was within $\pm 0.4\%$ of calculated values.

C. 3-Bromo-4-chloropyrazolo[3,4-d]pyrimidine, AVS 222



EXPERIMENTAL

3-Bromo-4-hydroxypyrazolo[3,4-d]pyrimidine (2)³: A mixture of 4-hydroxypyrazolo[3,4-d]pyrimidine (1), (150 g, 1.10 mol), bromine (748 g, 4.68 mol) and water (15 L) was heated at reflux for 67 hours. The product separated from the solution after standing at room temperature overnight. The light yellow crystals were collected by filtration, washed with water and air dried. Yield: 208.2 g (88.0%).

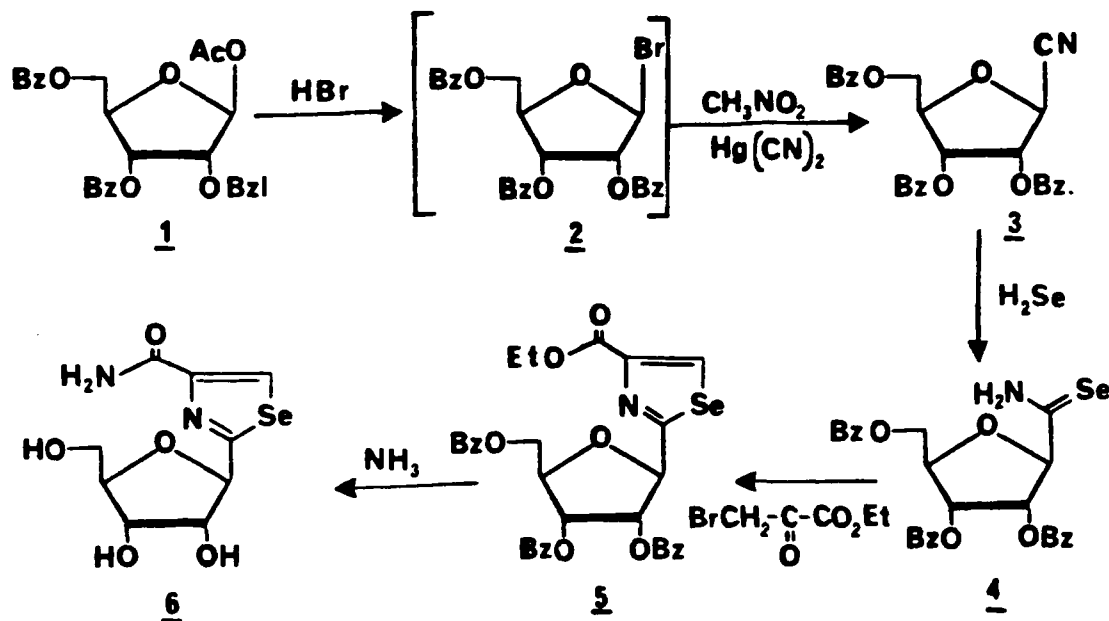
The procedure was repeated 2 times to give 415.1 g of product.

3-Bromo-4-chloropyrazolo[3,4-d]pyrimidine (3)⁴: Chlorinated product 2 (20.0 g, 0.093 mol), phosphorous oxychloride (250 mL) and diethyl aniline (30 mL) were combined and kept at reflux for 2 1/4 hours. Excess (150 mL) phosphorous oxychloride was removed under reduced pressure and the residue was poured on ice (500 g) while stirring. The aqueous mixture was extracted with ether (3 x 500 mL) and the combined ether layers were washed with ice water (500 mL), ice cold 2% sodium bicarbonate solution (500 mL) and ice water (500 mL). The organic phase was dried over sodium sulfate. The drying agent was filtered off and the organic solution containing 50 g silica gel was evaporated to dryness. The coated silica gel was loaded on a 200-425 mesh silica gel column and eluted with hexane/acetone (4:1) to give pure compound (3) upon evaporation of the solvent. Yield: 12.3 g (56.6%).

The procedure was repeated 7 times to give 64.6 g of final product.

D. 2-β-D-ribofuranosylselenazo-4-carboxamide, AVS 253

Synthetic Procedure:



EXPERIMENTAL

2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl-1-carbonitrile (3)⁵: A solution of 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribose (35.2 g, 0.07 mole) in toluene (140 mL) was cooled to -5°C . A slow stream of dry hydrogen bromide gas was passed into the solution for 1.5 hours maintaining the temperature at 0°C . The cooling bath was removed and the solution was allowed to warm to room temperature. After stirring at room temperature for 1/2 hour, dry nitrogen was injected into the solution to eliminate unreacted hydrogen bromide. The toluene was evaporated under reduced pressure at 40°C , then the residue was co-evaporated with toluene (2×100 mL). The residual oil formed a foam when placed under vacuum for 15

minutes. The foamy product was dissolved in nitromethane (100 mL), powdered mercuric cyanide (33.6 g, 0.133 mole) was added and the reaction mixture was stirred under anhydrous conditions at room temperature for 20 hours. The solid which separated from the reaction mixture was filtered and washed with toluene (3 x 100 mL). The filtrate and the washings were combined and evaporated to dryness. The resulting syrup was dissolved in ethyl acetate (400 mL) and extracted with a 5% potassium iodide solution (3 x 300 mL). The ethyl acetate layer was washed with 10% sodium hydrosulfide solution (150 mL). The black precipitate that formed was filtered through a celite bed and the celite bed was washed repeatedly with ethyl acetate. The two layers of the filtrate were separated and the organic layer was washed with water (3 x 300 mL), dried over anhydrous sodium sulfate and evaporated to yield a syrup which, on trituration with absolute ethanol, gave a white, crystalline product. The precipitate was filtered off, washed with ethanol and dried under vacuum. The mother liquor was concentrated and allowed to cool to give additional product (5.2 g). Yield: 23.8 g (72.3%). m.p. 80°C (Lit. m.p. 78-80°C). The spectral data of the product agreed with literature data.

The procedure was repeated four times with larger quantities to obtain enough material to be used in subsequent steps.

2,5-Anhydro-3,4,6-tri-O-benzoyl-D-allonselenoamide (4)⁶: The cyanosugar 3 (54.7 g, 0.116 mole) was dissolved in dry pyridine (550 mL) and cooled in an ice-salt bath to -5°C. A slow stream of hydrogen selenide gas was injected into the solution for 55 minutes (approx. 25 g, 0.31 mole). TLC (silica gel, benzene:ethyl acetate 4:1) indicated completion of the reaction. The reaction mixture was allowed to warm to room temperature. Dry nitrogen was bubbled through the reaction mixture to eliminate unreacted hydrogen selenide. The pyridine was evaporated under reduced pressure (bath temperature 35°C), toluene (2 x 200 mL) was added to the residue, and upon evaporation of the solvent, the resulting material was dissolved in ethyl acetate (400 mL). Charcoal (5.5 g) was added, and the mixture was stirred at room temperature for 1 hour. The charcoal was filtered through a celite bed and the bed was washed with ethyl acetate (3 x 100 mL). The red-colored filtrate was evaporated under reduced pressure at 35°C. The foamy product that formed was kept under high vacuum for 1 hour, then stored in the refrigerator. The product was homogeneous by TLC with a trace of α -anomer present. The spectral data of the product agreed with its structure. Yield 64.0 g (99.6%).

2-(2,3,5-Tri-O-benzoyl- β -D-ribofuranosyl)-4-ethoxycarbonylselenazole (5): The sugar selenamide 4 (63.9 g, 0.116 mol) was dissolved in dry acetonitrile (700 mL) and cooled in an ice bath to 0°C. A solution of ethyl bromopyruvate (35 g, 0.179 mole) in acetonitrile (200 mL) was slowly added through a dropping funnel while stirring. After the addition of the ethyl bromopyruvate, the reaction mixture was stirred at room temperature overnight. The solvent was evaporated under reduced pressure and the residue was treated with saturated sodium bicarbonate solution. Upon extraction with ethyl acetate (1400 mL), the organic layer was washed with water, dried over sodium sulfate and evaporated to give a dark-colored, syrupy residue. TLC of the product showed one major spot and several minor

spots. The syrup was dissolved in chloroform, combined with silica gel (100 g) to form a slurry, then the chloroform was evaporated. This coated silica gel was loaded onto a column packed with silica gel in hexane and eluted with a hexane-chloroform system. Fractions showing similar R_f values were combined and evaporated to yield 5 as a mixture of a and b anomers. Yield 52.0 g (69%).

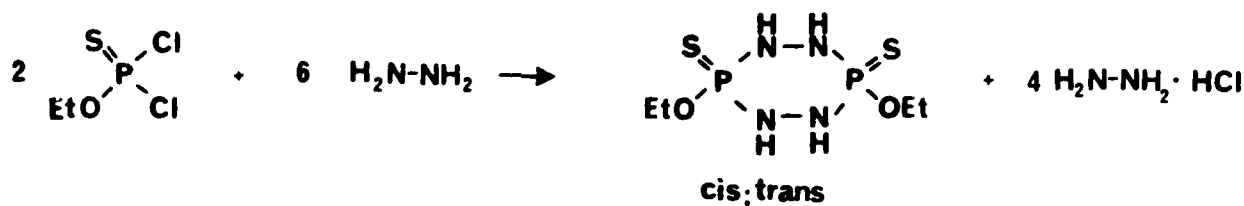
Since the chromatographic separation of the anomers was unsuccessful, the anomeric mixture was used in the subsequent reaction.

2- β -D-Ribofuranosylselenazo-4-carboxamide (6): The protected nucleoside 5 (49.0 g, 0.0756 mol) was dissolved in dry methanol (750 mL) and the solution was placed in a steel bomb. The bomb was cooled in a dry ice-acetone bath and liquid ammonia (approx. 120 mL) was added. The bomb was sealed and stirred at room temperature for 4 days. The bomb was cooled and opened. Excess ammonia evaporated as the contents warmed to room temperature. Silica gel (100 g) was added to the solution and the solvent was evaporated to dryness. The solid residue was treated with methanol (2 x 200 mL) to ensure proper coating of the compound onto silica gel. After evaporation the coated silica gel was loaded onto a column packed with silica gel (240-400 μ) in chloroform. Initial elution with chloroform gave benzamide and methyl benzoate as the byproducts. Subsequent elution with chloroform/methanol (9:1) produced various fractions. Fractions having identical R_f values were pooled and evaporated to dryness. The off-white solid that was obtained was dissolved in absolute ethanol, treated with charcoal, filtered and concentrated to 100 mL. Upon cooling, a crystalline solid separated. The solid was filtered, washed with cold ethanol and dried under vacuum. The mother liquor was again concentrated and cooled to produce an additional amount of product. Total yield 10.2 g (44%). m.p. 129-131°C (Lit. m.p. 131°C). Spectral properties and elemental analysis agreed with reported data.

The procedure was repeated 3 times to yield a total of 28.95 g of final product. During the later three batches, the final product had to be chromatographed three times to get the pure product, thus decreasing the yield significantly.

E. cis,trans 3,6-Diethoxy-tetradiphosphorino-3,6-disulfide, AVS 593

Synthetic Procedure:



Experimental

cis,trans 3,6-Diethoxy-tetrazadiphosphorino-3,6-disulfide⁷: To a solution of hydrazine monohydrate (65 g, 1.3 mol) in dry dioxane (540 mL) at 0°C was added dropwise a solution of ethyldichlorothiophosphate (80 g, 0.45 mol) dissolved in dry dioxane (200 mL). During addition the reaction mixture was stirred mechanically. Upon completion of addition the ice bath was removed, and the mixture was allowed to warm to room temperature.

The precipitated hydrazine hydrochloride was collected by filtration and the turbid filtrate was evaporated to dryness. The semi-solid residue was treated with chloroform (1 L), filtered, and the clear filtrate was evaporated to dryness. After dissolving the resulting solid in chloroform (400 mL) the slightly turbid mixture was left to stand overnight at -30°C, when the remaining hydrazine hydrochloride precipitated.

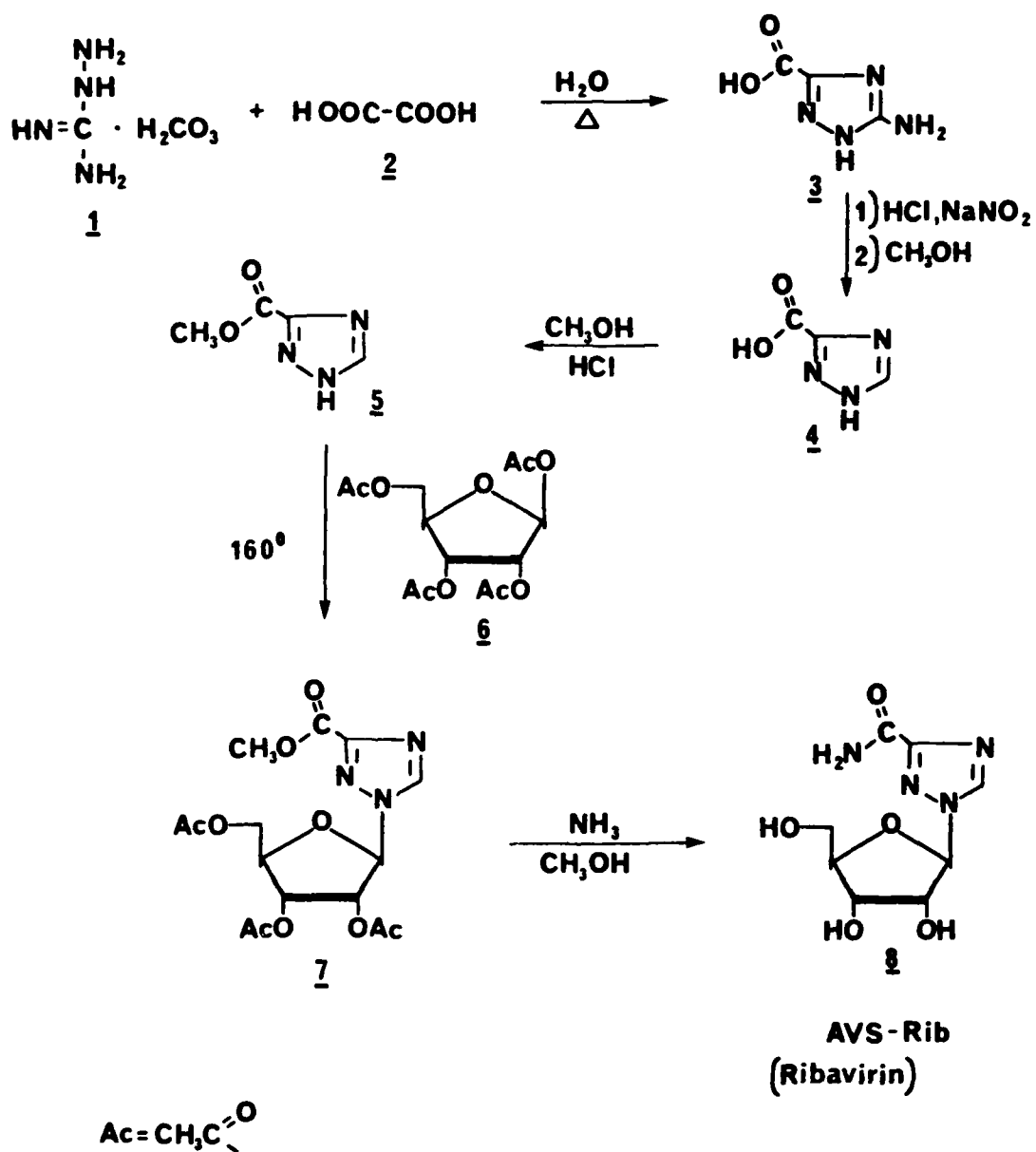
After filtration over a Celite bed the clear solution was taken to dryness under reduced pressure to yield 17 g of a white product, determined by TLC to be a crude mixture of the cis and trans isomers of AVS 593. After two column chromatographic separations on silica gel, with carbon tetrachloride/tetrahydrofuran 9:1 as the mobile phase, an isomeric mixture of crystalline AVS 593 was obtained. Yield: 10.7 g (8.6 %). Spectral data of the freshly prepared product agreed with published results, and elemental analysis was within ±0.3% of calculated values.

Remark: During thin layer chromatography on silica gel (carbon tetrachloride/tetrahydrofuran 9:1) the product tends to streak due to partial decomposition. Because of its relative instability the product should be stored under dry nitrogen.

Acknowledgement: We want to thank Professor Dr. Udo Engelhardt, Free University Berlin, for supplying the procedure for the synthesis of the title compound.

F. 1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide (Ribavirin), AVS RIB

Synthetic Procedure:



Experimental

5-Amino-1,2,4-triazole-3-carboxylic acid (3)⁸: Oxalic acid (11.12 Kg, 123.55 mol) was dissolved in 255 L water, while stirring aminoguanidine bicarbonate (10.5 Kg, 77.14 mol) was added portionwise. The reaction mixture was heated to 85°, and kept at 85-92° for eight hours. Upon cooling to about 70° a solution of sodium hydroxide (13.25 Kg, 50% in 25 L water) was added, then the reaction batch was reheated and kept at reflux for 90 minutes. After cooling overnight the turbid solution was filtered. The filtrate was neutralized with hydrochloric acid (3.2 L) and the resulting precipitate was collected by filtration. The product was dried at 55° while under aspirator vacuum (3 days). The product was used in the next step without further purification. Yield: 8.82 Kg (89%); m.p. 243° (lit. 242-244°)

1,2,4-Triazole-3-carboxylic acid (4)⁹: 5-Amino-1,2,4-triazole-3-carboxylic acid (1 Kg, 7.8 mol) was dissolved in hot hydrochloric acid (2.3 L conc. HCl and 6.4 L water). After cooling to 5°C a sodium nitrite solution (1.065 Kg sodium nitrite in 2.6 L water) was slowly added while maintaining the temperature below 10° by cooling in an ice bath. The precipitated diazo salt was collected by filtration and the filter cake was pressed dry without letting it go to complete dryness.

Caution: the dry diazocompound is explosive and it detonates violently when submitted to heat or friction.

A small amount of the moist diazonium salt was added to methanol (4 L at 35°), and upon initiation of the decomposition reaction strong cooling in an ice bath was required while maintaining the reaction in balance by adding small amounts of the diazonium salt. Upon completion the precipitated deamination product was collected by filtration and dried. Yield: 350 g m.p. 125-126°. This product was pure enough to be used in the subsequent esterification step.

Methyl-1,2,4-triazole-3-carboxylate (5)⁹: To a suspension of 1,2,4-triazole-3-carboxylic acid (1.8 Kg, 15.9 mol) in methanol (12 L) hydrochloric acid gas was injected while maintaining the temperature below 20° with cooling. After gas saturation the obtained solution was left at room temperature for five days. After that time the precipitated hydrochloride salt was collected by filtration, and dried, then added to water (4 L) for hydrolysis. The methyl ester was filtered and dried. The crude material, obtained from several batches (3205 g), was recrystallized from foiling water (25 L) to give purified crystalline methyl-1,2,4-triazole-3-carboxylate. Total Yield: 2560 g m.p. 198-199° (lit. 198°)

Methyl-1-(2,3,5-tri-O-acetyl- β -D-ribofuranosyl)-1,2,4-triazole-3-carboxylate (7)¹⁰: Methyl-1,2,4-triazole-3-carboxylate (127 g, 1 mol) and 1,2,3,5-tetra-O-acetyl- β -D-ribose (318 g, 1 mol) were mixed thoroughly and placed in a three-neck flask equipped with a mechanical stirrer, thermometer, and take-off condenser. The flask was immersed in an oil bath preheated to 165°. After the sugar derivative had melted bis-p-nitrophenylphosphate (2.5 g) was added. After stirring for 5 minutes the pressure in the reaction apparatus was reduced and the generated acetic acid distilled off. After 30 minutes the oil bath was removed, and the reaction mixture was left to cool to 50-60°.

AVS RIB

The highly viscous, dark reaction mass was slowly poured into cold methanol (1.2 L) while stirring, when the product started to crystallize.

Eighteen such fusion reactions were performed, and the combined batches were washed with methanol to give a total of 4.25 Kg (yield 61%) of methyl-1-(2,3,5-tri-O-acetyl- β -D-ribofuranosyl)-1,2,4-triazole-3-carboxylate. m.p. 104-105° (lit. 107-109°). Thin layer chromatography indicated that the product was almost exclusively the β -isomer.

1- β -D-Ribofuranosyl-1,2,4-triazole-3-carboxamide (Ribavirin) (g)¹⁰: Methanol (42 L) was placed in a stainless steel reactor and cooled to -10° by circulating alcohol at -40° through the reactor jacket. Liquid ammonia (8 L) was added to the cold methanol and the powdered acetylnucleoside 7 (4.24 Kg) was added in small portions. Upon completion of the addition the vessel was sealed air-tight, and the contents was allowed to warm up to room temperature. After two days the vessel was slowly vented and the contents was heated to 35-40°, then filtered through a filter pad. The filtrate was concentrated under reduced pressure, and the precipitated solid was collected by filtration to yield 2.1 Kg of crude ribavirin.

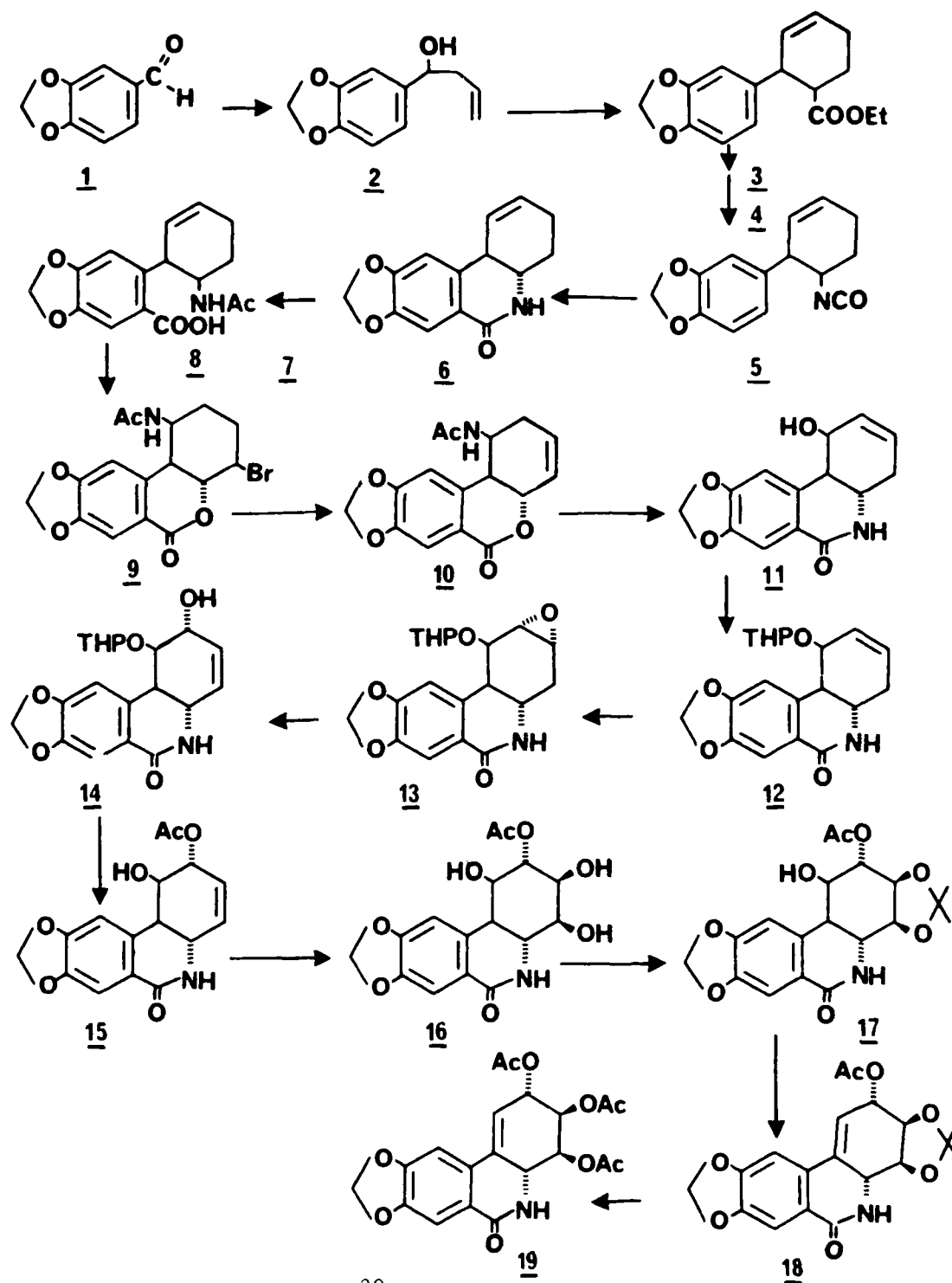
From the first and second mother liquor more product was obtained. Upon recrystallization from hot methanol-water 3:1 (13 L) a total yield of 2.34 Kg (88%) was obtained. m.p. 166-167° (lit. 166-168°).

Elemental analysis, thin layer chromatography, and spectral characteristics confirmed that the obtained product was the pure β -isomer of 1-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (Ribavirin).

VI. DISCUSSION OF UNCOMPLETED TARGET COMPOUNDS

G. Lycoricidine triacetate - AVS 360

The total synthesis of the alkaloid Lycoricidine¹³ has been reported by S. Ohta and S. Kimoto according to Scheme 1. In this twenty-one step procedure the reported overall yield was 2%, therefore the reaction sequence was initiated by preparing ca. 2 Kg of enol **2** to ultimately obtain the requested 5 grams of final product.



3,4-Methylenedioxyphenyl allyl carbinol (2)¹¹: 3,4-methylene dioxy-benzaldehyde (500 g, 3.33 mol) was dissolved in tetrahydrofuran (3 L) and the solution was cooled in an ice bath. Allyl magnesium chloride in THF (1000 mL, 2.0 mol) was added over a period of 2 1/2 hours while stirring. After allowing the reaction to warm to room temperature stirring was continued an additional three hours. Upon cooling a saturated solution of ammonium chloride (1 L) was added over a 1 hour period. The reaction mixture was extracted with chloroform (4 L), the organic layer was washed with sodium chloride solution (2 x 4 L), and the aqueous layer was washed with chloroform (1 L). The combined organic layers were dried with sodium sulfate, the solvent was evaporated under reduced pressure, and the remaining crude allyl carbinol 2 was used in the subsequent Diels-Alders reaction without further purification.

Ethyl-trans-2-(3',4'-methylenedioxyphenyl)-3-cyclohexane-1-carboxylate (3)¹²: Allyl carbinol 2 (300 g), ethyl acrylate (203.5 g) and p-toluene sulfonic acid (0.60 g) were heated in a sealed pressure vessel at 175-185°C for six hours. The unreacted ethyl acrylate was removed by vacuum distillation, and the residue was dissolved in ether (3 L). The ether layer was washed with water (1 L), 5% bicarbonate solution (1 L) and water (1 L). The organic phase was dried with magnesium sulfate, the ether was evaporated under reduced pressure, and the residue was submitted to fractional distillation, where the fraction distilling at 145-155°C/0.25mm Hg was identified as the ethyl ester 3.

After repeating the above described procedures four times with varying amounts but constant ratios of materials a total of 1304 g of ethyl-trans-2-(3',4'-methylenedioxyphenyl)-3-cyclohexene-1-carboxylate (3) was obtained.

trans-2-(3',4'-Methylenedioxyphenyl)-3-cyclohexenecarboxylic acid (4)¹²: A sodium ethoxide solution was prepared by dissolving sodium metal (10 g, 0.435 mol) in ethanol (300 mL). Ethyl ester 3 (100 g, 0.365 mol) was dissolved in ethanol (300 mL), and the solution was combined with the sodium ethoxide solution. After refluxing the reaction mixture for two hours, water (60 mL) was added, and refluxing was continued an additional four hours. The reaction mixture was left to stir at room temperature overnight. The solvent was evaporated under reduced pressure, and the residue was dissolved in water (1 L). The aqueous phase was washed with ether (3 x 300 mL), cooled in an ice bath, and adjusted to pH 1 with concentrated hydrochloric acid (25 mL). The resulting oil started to crystallize and the precipitate was collected by filtration, washed with water, and air-dried.

4H-r,3H-trans,4-Isocyano-3-(3',4'-methylenedioxyphenyl)cyclohex-1-ene (5)¹²: Carboxylic acid 4 (200 g, 0.81 mol) was dissolved in acetone (1 L), water (200 mL) and triethylamine (82 g, 0.81 mol) was added, and the solution was cooled in an ice bath. A solution of ethyl chloroformate (87.4 g, 0.81 mol) in acetone (200 mL) was added slowly while stirring. After two hours of continued stirring sodium azide (80 g, 1.23 mol), dissolved in water (200 mL) was added dropwise over a 1 hour period. While cooling, the reaction mixture was stirred an additional two hour period, then toluene (1 L) and water (1.5 L) was added and stirring was continued another 1.5 hours. The organic layer was separated, the aqueous layer was

extracted with toluene (300 mL), and the combined organic layers were washed with water (2 x 1 L), and dried (sodium sulfate).

The toluene solution was concentrated to 1700 mL under reduced pressure, then kept at reflux for four hours. After thin layer chromatography indicated the absence of starting material, the solvent was evaporated under reduced pressure to leave a light tan-colored oil, which was used in the next step without further purification.

4aH-r,10bH-trans,8,9-Methylenedioxy-3,4,4a,10b-tetrahydro-6(5H)-phenanthridone (6)¹²: Crude isocyano-derivative 5 (190 g) was kept under high vacuum for one hour while cooled in an ice bath. Boron trifluoride etherate (400 mL) was added carefully, and the reaction mixture was allowed to stand at room temperature overnight. The crystalline precipitate was collected by filtration, washed with ether (2 x 50 mL) and air-dried.

The filtrate was evaporated under reduced pressure, the residue was dissolved in ether, the solution was washed with water (3 x 400 mL), dried with sodium sulfate, the solvent was removed under diminished pressure and the residue was treated with boron trifluoride etherate (25 mL). After work-up, as described above, additional product was obtained. Total yield: 159.2 g $\hat{=}$ 80.9%

Repeating the above described procedure with varying amounts of isocyanoderivative 5 as the starting material a total yield of 864.3 g $\hat{=}$ 79.6% phenanthridone 6 was obtained.

41H-r,10bH-trans,5-Acetyl-8,9-methylenedioxy-3,4,4a,10b-tetrahydro-6(5H)-phenanthridone (7)¹²: A mixture of tetrahydrophenanthridone 6 (100 g, 0.411 mol), acetic anhydride (1600 mL), and p-dimethylaminopyridine (2.0 g, 8.4 mmol) was kept at reflux for 5 hours.

Excess acetic anhydride was evaporated under reduced pressure, and the residue was triturated with cold methanol (100 mL), the precipitated crystalline solid was filtered and washed with ethanol (2 x 100 mL). The product was air-dried, and thin layer chromatographic analysis indicated that the obtained acetyl-derivative 7 was suitable for the next step. Yield: 165 g (85%); m.p. 155-156; lit. 157-158.

Several individual runs were combined to give a total of 515 g of acetylated product 7.

4H-r,3H-trans-(3',4'-Methylenedioxy-6'-carboxyphenyl)-4-acetylamino-1-cyclohexene (8)¹³: To a suspension of N-acetylphenanthridone 7 in methanol (1 L) was added a solution of potassium hydroxide (22.4 g, 0.4 mol) in water (400 mL). The reaction flask was placed in a water bath at 80°, and the internal temperature reached 70°. After 15 minutes the reaction was allowed to cool to room temperature, when a white precipitate formed. The volume was reduced to 400 mL and the precipitate was collected by filtration. This precipitate was found to be unreacted starting material, and it was subsequently used in a future batch.

The filtrate was cooled in an ice bath, and the pH was adjusted to pH 1 with concentrated hydrochloric acid (25 mL). The resulting white

precipitate was filtered off, washed with water (2 x 25 mL) and air-dried. Thin layer chromatography and melting point agreed with reported values, hence the product was used in the next step without further purification. Yield: 40.0 g, m.p. 200-201°C; lit. 198-201°C.

4aH-r,1H-trans,10bH-cis,4-Htrans,1-Acetylamino-4-bromo-8,9-methylenedioxy-1,2,3,4,4a,10b-hexahydrodibenzo[b,d]pyrone-6 (9)¹³: To a well-stirred suspension of acetylamino carboxylic acid 8 (340 g, 1.12 mol) in dry tetrahydrofuran (4 L) powdered N-bromosuccinimide (204 g, 1.14 mol) was added in one portion. After several minutes most of the solid was dissolved and a crystalline material started to separate. After stirring for one hour the reaction flask was cooled in an ice bath and the precipitate collected by filtration. The filter cake was washed with cold tetrahydrofuran and dried in air. Upon concentrating the mother liquor additional product was obtained. Total yield: 400 g (93.5%); m.p. 275°; lit. 270-272°. From a smaller batch, 175.5 g was obtained.

4aH-r,1H-trans,10bH-cis,1-Acetylamino-8,9-methylenedioxy-1,2,4a,10b-tetrahydrodibenzo[b,d]pyrone-6 (10)¹³: A mixture of acetylamino bromolactone 9 (110 g, 0.28 mol), pyridine (1200 mL) and 1,8-diazobicyclo[5,4,0]undec-7-ene (44 g, 0.29 mol) was kept at reflux for 5 hours with exclusion of moisture. After standing overnight at room temperature the precipitated crystalline material was filtered off, and the filter cake was washed with pyridine (100 mL). The solid was stirred in water for five minutes, filtered, washed with water, and air-dried. From the mother liquor, upon concentration, more material was obtained. Total yield: 85.1 g (97.7%).

From an identical batch starting with 456.1 g bromolactone 9 339.0 g of product 10 was obtained, bringing the total yield to 424.1 g.

4aH-r,1H-trans,1-Hydroxy-8,9-methylenedioxy-1,4,4a,10b-tetrahydro-6(5H)-phenanthridone (11)¹³: Acetylamino lactone 10 (60 g, 0.19 mol) in ethanol (150 mL) was combined with a solution of sodium hydroxide (30 g) in water (150 mL). The reaction flask was immersed in a preheated oil bath at 95°C. The reaction mixture was stirred at 90-95°C for eight hours. Small amounts of water were added periodically to keep the reactants in solution. After eight hours the solution was allowed to cool to room temperature. The precipitated solid was collected by filtration, washed with water (3 x 50 mL) and air-dried.

The filtrate was cooled in an ice bath, neutralized with conc. hydrochloric acid, and the resulting precipitate was filtered, washed and dried. This second precipitate was identical to the first crop and therefore the two solids were combined. Yield: 48 g, m.p. 268-280°C; lit 265-280°C.

Three additional batches starting with 120 g of lactone 10 instead of 60 g resulted in a total yield of 332.9 g of hydroxyphenanthridone 11.

Presently the hydroxy group in phenanthridone 11 is being protected with dihydropyrane to enable further manipulations on the molecule, as outlined in the reaction scheme. It can be expected that the requested final product, lycoricidine triacetate (19), will become available within the next three months.

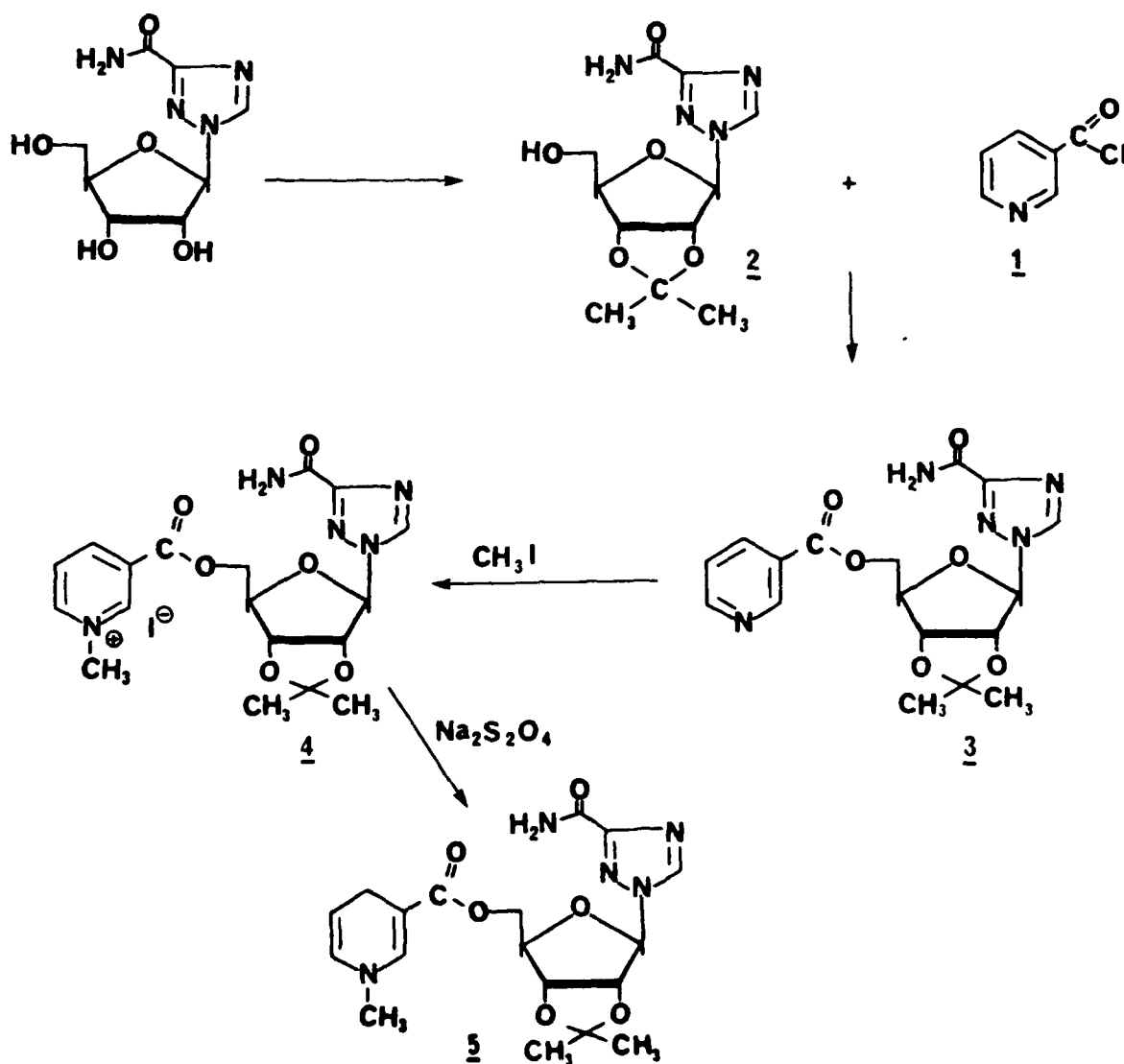
H. 5'-O-(1-Methyl-1,4-dihydronicotinoyl)-1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (Prodrug ester), AVS XXX:

The preparation of AVS XXX was approached by various strategies, however, each one of the methods ultimately failed to yield the desired product.

The use of dehydrating agents such as dicyclohexylcarbodiimide (DCC)¹⁴ or trimethylsilyl chloride¹⁵ to couple the nicotinoyl, or the N-methyl nicotinoyl moiety to the 5'-position of ribavirin did not produce the desired ester, but resulted in the formation of some dehydration products.

It is possible to mask the carboxyl group of nicotinic acid by converting it into an oxazoline derivative¹⁶ but subsequent coupling of the reduced N-methyl derivative with ribavirin failed.

It was possible to obtain the 2',3'-isopropylidene derivative of AVS XXX according to the following scheme:



Nicotinoylchloride (1)¹⁷: To potassium nicotinate (258 g, 1.6 mol) suspended in dry benzene (1150 mL) and cooled in an ice bath a solution of oxalyl chloride (200 g, 1.58 mol) in dry benzene (380 mL) was added dropwise. After addition stirring was continued for 20 minutes then the reaction was allowed to warm to room temperature. The reaction was kept at reflux for 30 minutes, cooled, and the precipitated potassium chloride was collected by filtration. The filtrate was concentrated to an oil under reduced pressure, and the product was purified by fractional distillation. Yield: 148.5 g (59%); b.p. 128-133°/80-90 mm Hg.

2',3'-O-Isopropylidene-ribavirin (2)¹⁸: Ribavirin (75 g, 0.31 mol) was stirred in a mixture of acetone (1 L) and dimethoxypropane (500 mL). Upon cooling perchloric acid (30 mL) was added dropwise over a five minute period. Stirring was continued while the reaction warmed up to room temperature. After 3½ hours the orange-yellow solution was neutralized with 2N potassium hydroxide (200 mL), the turbid mixture was filtered over Celite and the filtrate was evaporated to dryness. The solid residue was stirred in methanol (2 L) and filtered. The filtrate was concentrated to a slurry by distillation under reduced pressure, the precipitate was collected by filtration and the filter cake was recrystallized from ethyl acetate (1 L)/methanol (250 mL). Yield: 66 g (75.6%); m.p. 151°C, lit. 152-154°C.

5'-Nicotinoyl-2',3'-O-isopropylidene-ribavirin (3): Isopropylidene ribavirin 2 (7.1 g, 25 mmol) was placed in dry pyridine (60 mL) and the mixture was cooled in an ice bath. While stirring nicotinyl chloride (10.5 g, 65.4 mmol), dissolved in chloroform (30 mL) was added. The reaction mixture was left to stir at room temperature overnight. Three drops of water were added to the solution, followed by chloroform (200 mL). The organic layer was washed with water (200 mL), bicarbonate solution (200 mL) and water. After drying with sodium sulfate, the chloroform layer was evaporated to dryness, then coevaporated three times with toluene (150 mL) to leave a highly viscous, pyridine-free oil. Yield: 9.6 g.

Spectral and chromatographic analyses confirmed the structural assignment, and the product was used in the following methylation step without further purification.

5'-(N-Methyl-nicotinoylium)-2',3'-O-isopropylidene-ribavirin iodide (4): Nicotinoyl ester 4 (8.5 g) was dissolved in dry acetone (30 mL), methyl iodide (5 g) was added and the solution was refluxed. After 30 minutes thin layer chromatography indicated the disappearance of starting material 3, and the solvent was evaporated under reduced pressure. The residual yellow solid foam was extremely hygroscopic, and it changed into a highly viscous mass when exposed to atmospheric moisture. Yield: 6.2 g. This material was immediately used in the subsequent reduction reaction.

5'-(N-Methyl-1,4-dihydronicotinoyl)-2',3'-isopropylidene-ribavirin (5): Nicotinoyl ester derivative 4 (6.2 g, 11.3 mmol) was dissolved in deaerated water (200 mL), followed by the addition of sodium bicarbonate (6 g). The yellow solution was cooled in an ice bath, while sodium dithionate (8 g) was added over a 15 minute interval. A yellow, gummy precipitate formed and the reaction was left to warm to room temperature. The aqueous layer was extracted with ethyl acetate (4 x 200 mL), the organic layer was

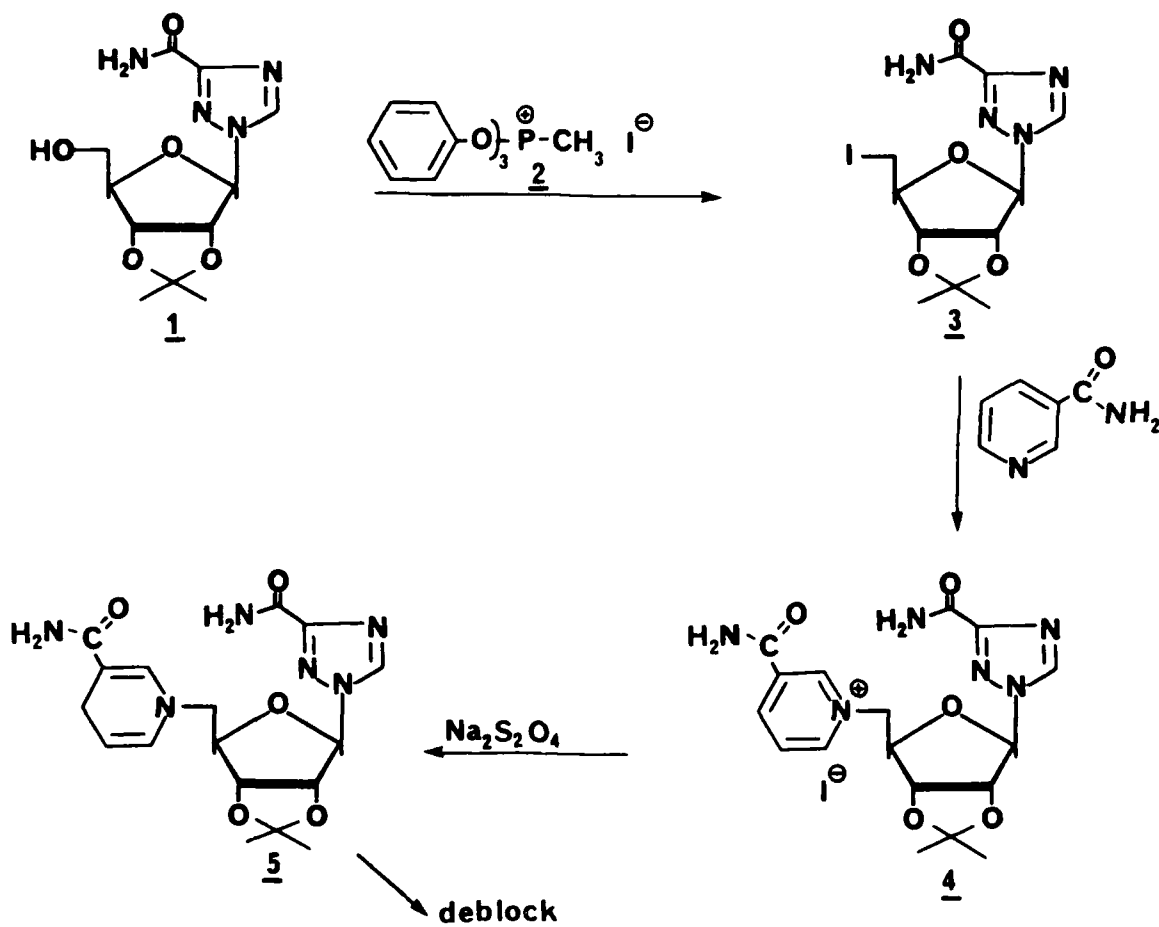
dried (sodium sulfate) and evaporated, leaving a highly viscous, yellow liquid. Nuclear magnetic resonance and infra-red spectral analyses indicated the presence of the desired reduction product as the major component, however, thin layer chromatographic examination showed the presence of several products.

After isolating the major component by preparative thin layer chromatography as a single band, and eluting the product from the support, the resulting solution again contained several byproducts, as shown by thin layer chromatography. A repetition of the same procedure always produced the same result, indicating that the desired product was extremely unstable during purification and isolation processes.

Attempts to prepare AVS XXX and analogues, such as the 2',3'-diacetyl derivative will be continued, where special care will be taken to shield the air-sensitive intermediate and final products from exposure to oxygen and moisture.

I. 5'-Deoxy-5'-(N-1,4-dihydronicotinamidyl)-1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide, AVS SPEC

Synthetic Procedure:

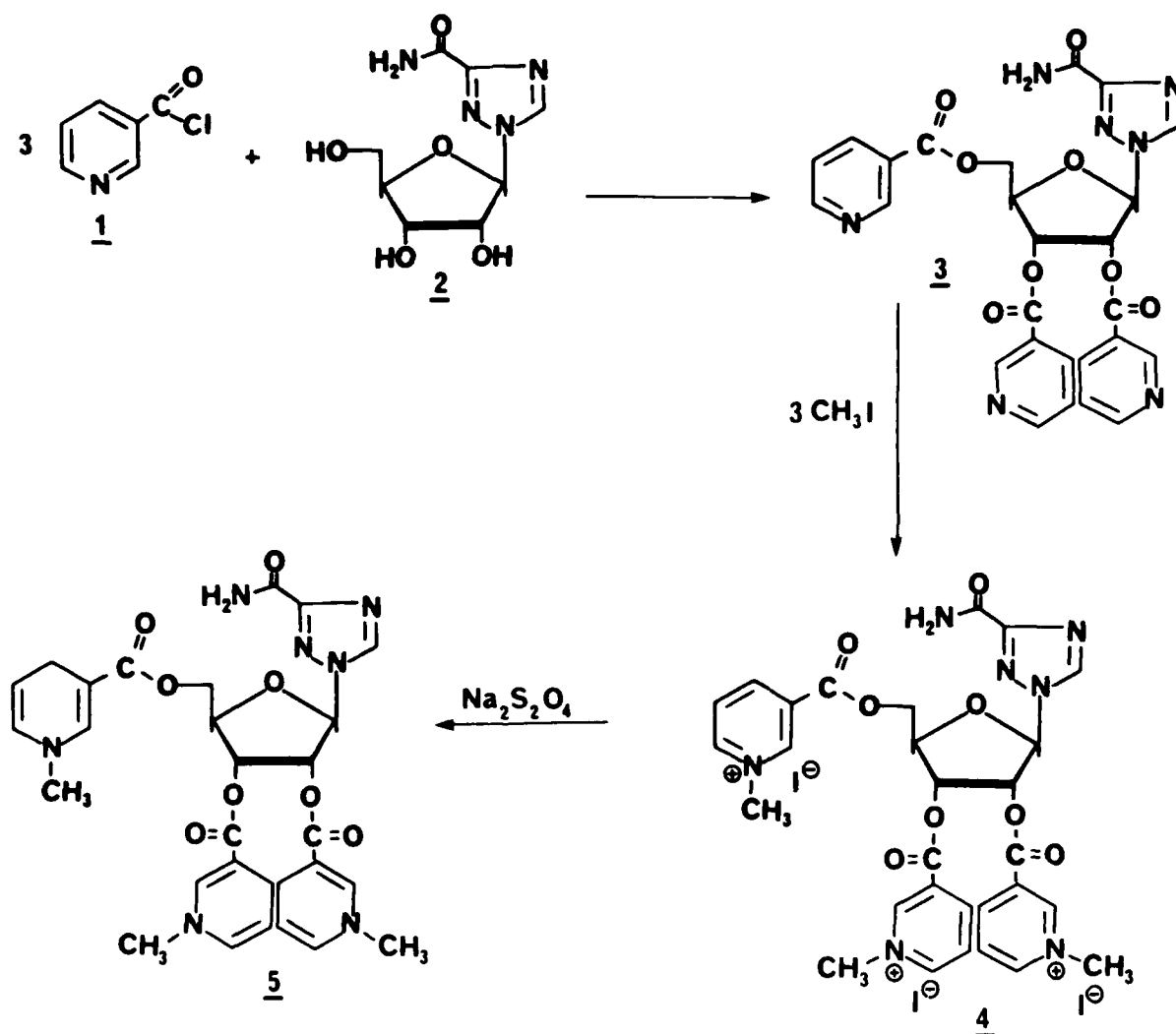


5'-deoxy-5'-iodo-2',3'-O-isopropylidene-ribavirin (3)¹⁹: Under an atmosphere of dry nitrogen methyltriphenoxyphosphonium iodide (2) (27.9 g, 61.7 mmol) was added to a solution of isopropylidene ribavirin 1 (8.7 g, 30.6 mmol), dissolved in 150 mL dry dimethylformamide. After standing at 25° for one hour the solvent was evaporated under high vacuum, the residue was dissolved in ethyl acetate (250 mL), and hexane was added dropwise until a precipitate appeared. The amorphous crystals were collected by filtration and dried. Yield: 5.2 g. Analytical and chromatographic data agreed with reported values for the iodo-ribavirin derivative 3.

The next step will be the N-alkylation of nicotinamide with iodoribavirin to form the quaternary salt 5.

K. 2',3',5'-Tri-O-(1-methyl-1,4-dihydronicotinoyl)-1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide, AVS TRI

Synthetic Procedure:



2'3'5'-Tri-O-nicotinoyl-ribavirin (3): Ribavirin (2) (7.3 g, 30 mmol) was placed in pyridine (70 mL), the mixture was cooled, and nicotinoyl-chloride (1) (17.0 g, 120 mmol), dissolved in chloroform (60 mL) was added portionwise. After stirring one hour the reaction mixture was heated to reflux, and kept at reflux for eight hours. Thin layer chromatography indicated the presence of di- and tri-acylated ribavirin.

New methods will be studied to bring the acylation reaction to completion. The tri-acylated product will be methylated at the pyridyl nitrogens, followed by reduction to give the lipid-soluble ribavirin derivative 5.

VI. REFERENCES

1. A. Yamane, A. Matsuda and T. Ueda, Chem. Pharm. Bull., 1980, 28, 150.
2. J. D. Westover, G.R. Revankar, R.K. Robins, R.D. Madsen, J.R. Ogden, J.A. North, R.W. Mancuso, R.J. Rousseau, and E.L. Stephen, J. Med. Chem., 1981, 24, 94.
3. Chu, I.; Lynch, B.M. J. Med. Chem. 1975, 17, 161-5.
4. Cottam, H.B. et. al. J. Med. Chem. 1984, 27, 1119-27.
5. M. Bobek and J. Farkas, Coll. Czech. Chem. Commun., 34, 247 (1969).
6. P.C. Srivastava and R.K. Robins, J. Med. Chem., 26, 445 (1983).
7. Viertel, H. Dissertation, Freie Universitat Berlin, Berlin F.R.G. 1981. Personal Communication: Professor Dr. U. Engelhardt, Freie Universitat Berlin.
8. Chipen, C. Chem. Abst. 1967, 67, 64306V.
9. Chipen, G.I.; Grinshtein, V. Ya. Chem. Heterocycl. Compounds (USSR) 1965, 1, 420.
10. Witkowski, J.T.; Robins, R.K.; Sidwell, R.W.; Simon, L.N. J. Med. Chem. 1972, 15, 1150-54.
11. Quelet, R.; Drau, R. Compt. Rend., 1964, 258, 1826.
12. Ohta, S.; Kimoto, S. Chem. Pharm. Bull. (Japan), 1976, 24, 2969-76.
13. Ohta, S.; Kimoto, S. ibid, 1976, 24, 2977-84.
14. Angew. Chem. Int. Ed. 1978, 17, 522.
15. Synthesis 1983, 3, 201-3.
16. Dubey, S.K.; Knaus, E.F. Can. J. Chem. 1985, 63, 559-64.
17. Wingfield, Jr., H.N.; Harlan, W.R.; Hammer, H.R. J. Am. Chem. Soc. 1953, 75, 4364.
18. Fuertes, M.; Witkowski, J.T.; Streeter, D.G.; Robins, R.K. J. Med. Chem., 1974, 17, 642-5.
19. Verheyden, J.P.H.; Moffat, J.G. J. Org. Chem. 1970, 7, 2319-26.

VII. ACKNOWLEDGEMENTS

The personnel assigned to this contract during the past annual period were: Ernst M. Schubert, Ph.D., Principal Investigator; Bheemaroo Ugarkar, Ph.D., Principal Assistant; Jay DaRe, B.S., Chemist; Penny Domenico, B.S., Chemist, from January 1, 1985 to June 30, 1985.

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Pharm-Eco Laboratories, Inc.

A handwritten signature in cursive script, appearing to read "Ernst Schubert", is written over a horizontal line.

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